the basis for fitting the αIIb β-propeller and the β3 βI and PSI domains into the map. Reasoning that the electron density adjacent to the bilayer must fit distal segments of the integrin legs, we steered the corresponding crystallographic regions towards the section of the map immediately above the nanodisc. The adjacent β3 EGF-4 and EGF-3 domains were steered towards the higher density regions in the lower part of the map, whereas a final MDFF run allowed optimal fitting of the β3 EGF-1 and EGF-2, and the αIIb thigh domains into the remaining electron density. Our predicted model structure of the αIIbβ3 ectodomain shows that the headpiece is oriented away from the lipid bilayer and, in sharp contrast to the crystal structure, that the lower legs are not straight, parallel, and adjacent; rather they show definite bends and join only when near the bilayer.

363-Pos Board B132

Phosphorylated Phospholamban Stabilizes a Distinct Compact Conformation of Sarco(endo)Plasmic Reticulum Ca-ATPase

Sandeep Pallikkuth, **Daniel Blackwell**, Dane Zieman, Seth Robia.
Loyola University Chicago, Maywood, IL, USA.

Sarco(endo)plasmic reticulum Ca-ATPase (SERCA) is inhibited by the 52 amino acid protein phospholamban (PLB). Inhibition of SERCA is relieved when PLB is phosphorylated by protein kinase A (PKA). To better understand the mechanism of this regulatory mechanism on SERCA structure, we designed a ''2-color'' SERCA labeled with donor and acceptor fluorescent proteins in the A- and N-domain. Intramolecular fluorescence resonance energy transfer (FRET) between these two domains was quantified as an index of SERCA conformation. Fluorescence lifetime distribution analysis (FLDA) showed that SERCA sampled three discrete conformations in the absence of PLB or in the presence of non-phosphorylatable PLB (S16A). Co-transfection of SERCA with phosphomimetic PLB (S16E) revealed an additional state characterized by a highly compact conformation. This unique state was only observed under conditions of high intracellular calcium. The data support the hypothesis that PLB remains bound to SERCA after phosphorylation by PKA, inducing the pump to assume a very compact E1 conformation.

364-Pos Board B133

Dynamic Motions of G Proteins during Receptor Independent Cell Signaling

Labe Black, Gwen Nix, Celestine Thomas, Michelle Terwilliger,
Stephen Sprang, J.B. Alexander Ross.
University of Montana, Missoula, MT, USA.

The Gα subunit is responsible for regulating intracellular signals through guanine nucleotide binding and release, the catalytic rate limiting step. Recent studies have shown interactions of Gα with Guanine nucleotide Exchange Factors (GEFs) cause dynamic structural changes which facilitate substrate release. Determining the structural changes and dynamics of the GDP-bound (inactive) and GEF-bound (nucleotide-free) states of the α-subunit will lead to a better understanding of cell signaling and regulation. In this study the conformational state distributions and dynamic motions of Gα were probed on a surface-cysteine free Gαi1 construct (Gα Hexa I) mutant using single molecule Förster Resonance Energy Transfer (smFRET)and time-resolved fluorescence anisotropy.

365-Pos Board B134

Structure Transitions of the Sodium, Potassium-ATPase Investigated by Intramolecular FRET

Alexander L. Ticho[1], Neha Abrol[1], Julie Bossuyt[2], Donald M. Bers[2],
Seth L. Robia[1].
[1]Loyola University Chicago, Maywood, IL, USA, [2]University of California - Davis, Davis, CA, USA.

The sodium, potassium-ATPase (NKA) is an important ion pump responsible for the generation of electrochemical gradients across eukaryotic plasma membranes. This protein is a high-value therapeutic target for the treatment of heart failure and several carcinomas. We fused fluorescent proteins to the N and A domains of NKA and investigated conformational changes by quantifying intramolecular FRET. Fluorescence lifetime distribution analysis (FLDA) revealed a decrease in FRET with the addition of 25 mM KCl or 1mM ouabain, suggesting a structure transition to a more open conformation. In addition, the low-FRET conformations show a wider lifetime distribution than high-FRET architectures, which suggests that the open structure has a more dynamic cytoplasmic headpiece than the closed conformation. Ongoing experiments will investigate the voltage dependence of FRET changes and NKA structural heterogeneity.

366-Pos Board B135

Single Molecule Förster Resonance Energy Transfer Studies of the Fc Region of a Human Antibody

Cathrine A. Southern, Michael T. Kelliher, Madeline E. Gemoules,
Mark S. Piraino, Ramiah D. Jacks, Ashley E. Hall.
DePaul University, Chicago, IL, USA.

The crystallizable fragment (Fc) region of IgG antibodies interacts with a variety of molecules in the immune system. The removal of the oligosaccharides bound to this region affects the ability of antibodies to generate an immune response. A comparison of the structures of the Fc region with and without the oligosaccharides present can indicate if a structural change is the basis for this effect. To carry out these studies, we have labeled the Fc region of a human IgG antibody with donor and acceptor dye molecules. Enzymatic removal of the oligosaccharides was used to generate an oligosaccharide-free antibody. Both ensemble and single molecule Förster resonance energy transfer (FRET) spectroscopy were used to examine the structures of the Fc region. All single molecule FRET measurements performed involved the examination of photon bursts from freely diffusing donor-acceptor labeled antibodies, from which a histogram of the conformations present was constructed.

367-Pos Board B136

The Effect of Urea on the Activity and Allosteric Coupling in Phosphofructokinase from Bacillus Stearothermophilus and Escherichia Coli

Amy M. Whitaker, Gregory D. Reinhart.
Texas A&M University, College Station, TX, USA.

Phosphofructokinase (PFK) catalyzes the phosphorylation of fructose-6-phosphate (Fru-6-P) by MgATP in the first committed step of glycolysis. Bacterial phosphofructokinase is an allosteric, homotetrameric enzyme and is allosterically regulated by phospho(enol)pyruvate (PEP) and MgADP. In phosphofructokinase from both Bacillus stearothermophilus (BsPFK) and Escherichia coli (EcPFK) the allosteric coupling between PEP and Fru-6-P results from compensating enthalpy and entropy components. In EcPFK the positive coupling free energy associated with inhibition by PEP is established by the positive enthalpy component. In BsPFK the sign of the coupling free energy is opposite that of the enthalpy term and is therefore determined by the larger absolute value of the entropy term. This striking contrast between BsPFK and EcPFK suggests that fundamentally different mechanisms may be responsible for transmitting the allosteric signal between binding sites. In the present study, enzyme activity and allosteric couplings were determined at varying concentrations of urea for both BsPFK and EcPFK. The specific activity of BsPFK increases at low concentrations of urea, reaching 115% of initial activity at ~0.5M urea. The activity remains at this level until ~2.5M urea, where it decreases until reaching 0% at ~6M urea. EcPFK slowly loses activity from 0 to ~1.5M urea, where the activity begins to quickly decline and is completely lost by ~3M. Compared to 0M conditions, the allosteric coupling between PEP and Fru-6-P increases by about 3-fold in 0.5M urea, 2-fold in 2M urea, and decreases by 2-fold in 4M urea. This is in direct contrast to EcPFK, where the allosteric coupling is similar at 0, 0.5M and 2M urea. These results support the notion of different underlying mechanisms for inhibition of PFK in Bacillus stearothermophilus and Escherichia coli. Funding: NIH-GM33261, NIH-CBI, Welch-A1543.

368-Pos Board B137

Allosteric Modulation of Cadherin Affinity

Nitesh Shashikanth[1], Jillian Newhall[1], Yuliya I. Petrova[2],
Barry M. Gumbiner[2], Deborah Leckband[1].
[1]University of Illinois Urbana Champaign, Urbana, IL, USA, [2]University of Virginia, Charlottesville, VA, USA.

We report direct evidence for the allosteric regulation of cadherin adhesion by conformational changes and inside-out signaling. Cadherins are essential proteins that mediate intercellular cohesion in all tissues, and circumstantial evidence suggested that inside-out signaling allosterically regulates cadherins, analogous to integrins. Recent findings with conformationally-specific antibodies provided biochemical evidence that cadherin adhesion is allosterically modulated by conformational changes in association with a conformational change in the cadherin cytoplasmic domain and in the phosphorylation state of a cytoplasmic binding protein, p120 catenin. Here we present direct evidence, using micropipette manipulation that antibody-induced conformational changes alter the intrinsic affinity of membrane-bound cadherins. Micropipette measurements quantified the two-dimensional affinity and dissociation rate of bonds between cell surface cadherins. Studies used Colo205 cells, which express a full complement of surface E-cadherin, but do not aggregate unless treated with a kinase inhibitor or with activating antibodies. Binding kinetics between Colo205 cells and cadherin-displaying red blood cells showed that activating monoclonal antibodies substantially increase the intrinsic affinity of membrane-bound cadherin. Neutral antibodies, which bind cadherin but do not alter Colo205 aggregation, had no effect on cell-cell binding. These important findings demonstrate conformational coupling between cadherin binding activity, extracellular domain conformations, and intracellular signaling.